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Effects of deltamethrin on photosynthetic pigments and ascorbate-glutathione (ASA-GSH) cycle in *Lemna minor*

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Abstract: Deltamethrin is a synthetic pyrethroid insecticide that can cause adverse effects on non-target organisms. This study was designed to investigate the effects of different concentrations (0.001, 0.005 and 0.01 ppm) of deltamethrin on photosynthetic pigments and the ascorbate-glutathione (ASA-GSH) cycle in *Lemna minor*, a freshwater macrophyte. To assess the effect of deltamethrin on *L. minor*, photosynthetic pigments, malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) levels, and the activities of some antioxidant enzymes (SOD, CAT and POD) and enzymatic and non-enzymatic antioxidants associated with the ASA-GSH cycle were measured. The results showed that exposure to deltamethrin decreased chl a, chl b and carotenoid levels and increased MDA and H_2O_2 levels. In addition, deltamethrin exposure significantly increased SOD, CAT and POD activities. The activities of ASA-GSH cycle enzymes (APX, GR, GPX, MDHAR and DHAR) decreased in *L. minor* exposed to 0.01 ppm deltamethrin, while GST activity increased. Exposure to low doses of deltamethrin increased ASA and GSH levels, while 0.01 ppm deltamethrin decreased the amounts of ASA and GSH compared to the control. Taken together, the present study revealed that different concentrations of deltamethrin inhibited photosynthetic activity, increased lipid peroxidation and caused oxidative stress and activated the antioxidant defense system of *L. minor* to eliminate the increased oxidative stress.

Keywords: Antioxidant enzyme; Ascorbate, Deltamethrin; Glutathione; *Lemna minor*; Malondialdehyde

1 Introduction

Deltamethrin is a synthetic pyrethroid insecticide and a powerful insecticidal chemical widely used in many fields such as agriculture, animal husbandry and public health. Obtained as a result of chemical modifications of natural pyrethrins, deltamethrin causes insects to die quickly, mainly due to its effects on the nervous system (Lu et al. 2019). By blocking sodium channels in nerve cells, it disrupts insects' nerve conduction, leading to paralysis resulting in death. Deltamethrin is also used to protect fish from ectoparasites (Abdelhalak et al. 2015). In India and some other developing countries, deltamethrin has been reported to be used to control mosquitoes carrying zika virus and dengue virus (WHO, 2010). Synthetic pyrethroids such as deltamethrin have high insecticidal activity and low toxicity to mammals and birds (Lu et al. 2019). Since the implementation of organophosphorus insecticide sales restrictions, the use of pyrethroid insecticides such as deltamethrin has increased significantly and pyrethroid insecticides have become the preferred choice in many agriculture-based countries over the past two decades (Kumar et al. 2016). However, due to their widespread use, pyrethroid insecticide contamination has become a major problem worldwide. Deltamethrin-contaminated waters have started to harm aquatic biota, and the consumption of food contaminated with this insecticide has become a danger to animal and human health (Barlow et al. 2001). While deltamethrin was initially thought to have low toxicity to mammals, several studies have reported toxic effects of this insecticide on non-target organisms (Lu et al. 2019). Deltamethrin has been reported to increase the amount of reactive oxygen species, cause oxidative stress, immunotoxicity and neurotoxicity in various organisms (Lu et al. 2019). Since the consumption of deltamethrin-treated foods may affect human health in the long term, further research on the toxicity of this pesticide is needed. In this study, the effect of deltamethrin on the antioxidant system of *L. minor*, a non-target aquatic organism, was evaluated. *L. minor* is a monocotyledonous plant of the Lemnaceae family

that grows in nutrient-rich freshwater. In addition to being food for fish and poultry, they have economic potential due to their small size and rapid reproduction and are used in research in biotechnology and ecology (Alp et al. 2023). Previous studies have shown that *L. minor* is sensitive to various environmental contaminants such as heavy metals and pesticides (Li et al. 2022). Therefore, *L. minor* has been used as a biomonitor to assess the ecotoxicity of various chemicals to the aquatic environment (OECD, 2006).

In this study, the effect of deltamethrin, which is widely used both in the world and in our country, on *L. minor*, a non-target organism, was evaluated. In the study, 3 different concentrations of deltamethrin were applied to *L. minor* and the changes in photosynthetic pigments, lipid peroxidation, hydrogen peroxide and enzymatic and non-enzymatic antioxidants related to the antioxidant system were analyzed.

2 Materials and Method

The duckweed (*Lemna minor* L.) used in this study was collected from the wetlands around Erzurum airport. The collected plants were stressed with 10% NaClO and 1% $HgCl_2$ for 2 minutes and then washed several times with pure water. The plants were cultured in 1/10 Hoagland's solution for 3 months in the Plant Physiology Laboratory of Atatürk University, Faculty of Science, Department of Biology. Toxicity testing was carried out according to the OECD guidelines. For the experiments, 600 healthy *L. minor* plants were selected. Approximately 150 (50X3) plants of the same size were used for each test group. The experiments were carried out in 50 ml Petri dishes. Each Petri dish was filled with 50 ml of 10% Steinberg's solution and 50 *L. minor* plants. The experiments were carried out in the acclimatization room of Atatürk University, Department of Biology, at a temperature of 24±2 °C, 16/8 light/dark photoperiod and 60% humidity. Three different concentrations of deltamethrin, 0.001, 0.005 and 0.01 ppm, were used in the experiments. The concentrations used in the experiments were determined as a result of preliminary experiments. While only 50 ml of 10% Steinberg solution was added to the control group, deltamethrin was added to the Steinberg solution in the other experimental groups. The experiments were carried out in 3 parallel experiments. Plants were harvested after 7 days and used for analysis.

The procedure recommended by Witham et al. (1971) was used to determine chlorophyll a, chlorophyll b and carotenoid content.

To determine lipid peroxidation in L. minor plant treated with tetraconazole, the method proposed by Velikova et al. (2000) was used. In order to determine the amount of hydrogen peroxide (H_2O_2), the determination was carried out by making some modifications to the method of Velikova et al. (2000).

Superoxide dismutase (SOD) activity was determined by the method of Agarwal and Pandey (2004) with minor modifications, catalase (CAT) activity was determined by observing the decomposition of H_2O_2 according to the method of Aebi (1984); peroxidase (POD) activity was measured by monitoring the oxidation of guaiacol in the presence of H_2O_2 according to the method of Yee et al. (2002), and ascorbate peroxidase (APX) activity was measured based on the decrease in absorbance at 290 nm according to the method of Nakano and Asada (1981). Glutathione reductase (GR) activity was analyzed by a modified method based on the protocol of Foyer and Halliwell (1976). Glutathione S-transferase (GST) activity was determined according to the method of Habig et al. (1974). Glutathione peroxidase (GPX) activity was determined et 470 nm based on the method described by Hasanuzzaman et al. (2012). Monodehydroascorbate reductase (MDHAR) activity was measured by the method of Miyake and Asada (1992) with minor modifications. Dehydroascorbate reductase (DHAR) activity was measured based on the method of Nakano and Asada (1981) with some modifications. Total ascorbate and GSH were measured using the methods proposed by Huang et al. (2005) and Yu et al. (2003), respectively, with some modifications. Briefly, 0.5 g tissue was grounded in 5% meta-phosphoric acid and 1mM EDTA, then centrifuged 12000 rpm for 10 min. Obtained supernatant was used for analysis ASA and GSH content.

The experiments were repeated three times and the results are presented as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) was used to analyze the data and Dunnett's multiple comparison tests were applied. Significance limits were set as *P < 0.05, **P < 0.01, ***P < 0.001 and ****P < 0.0001. All statistical analyses were performed with GraphPad Prism 8.4 software.

3 Results and Discussion

Chlorophyll a, chlorophyll b and carotenoid amounts showed a decreasing trend in parallel with the increase in deltamethrin concentration (Fig. 1). Compared to the control group, the chlorophyll a content of *L. minor* decreased by approximately 50% in the 0.01 ppm treated group. Chlorophyll b content decreased by approximately 36% in the 0.01 ppm treated group compared to the control group. Carotenoid content decreased by approximately 50% in the 0.01 ppm group compared to the control group. In parallel with these results, the inhibitory effect of deltamethrin on chlorophyll a, chlorophyll b and carotenoid pigments was also shown in maize, soybean and tomato plants (Bashir et al. 2007; Duran et. al. 2015; Touzout et al. 2021).

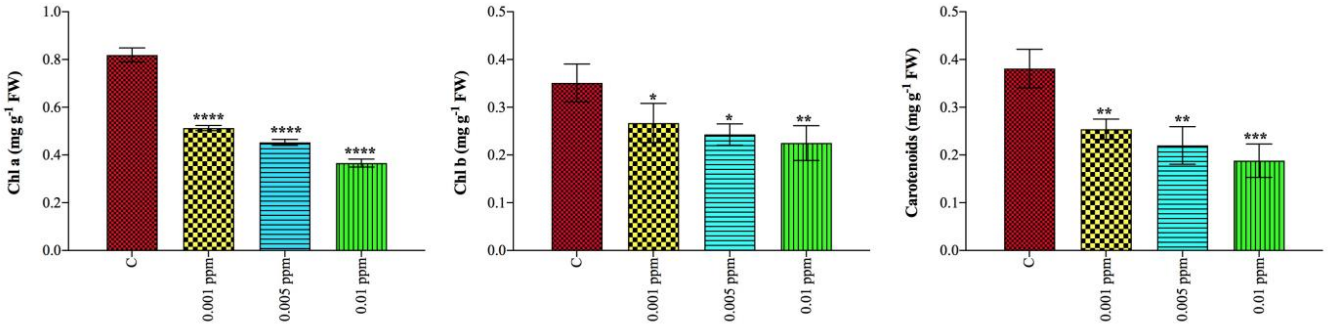

Fig. 1 Effect of deltamethrin on photosynthetic pigments in *L. minor*. Values are given as mean ± S.D. *p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001.

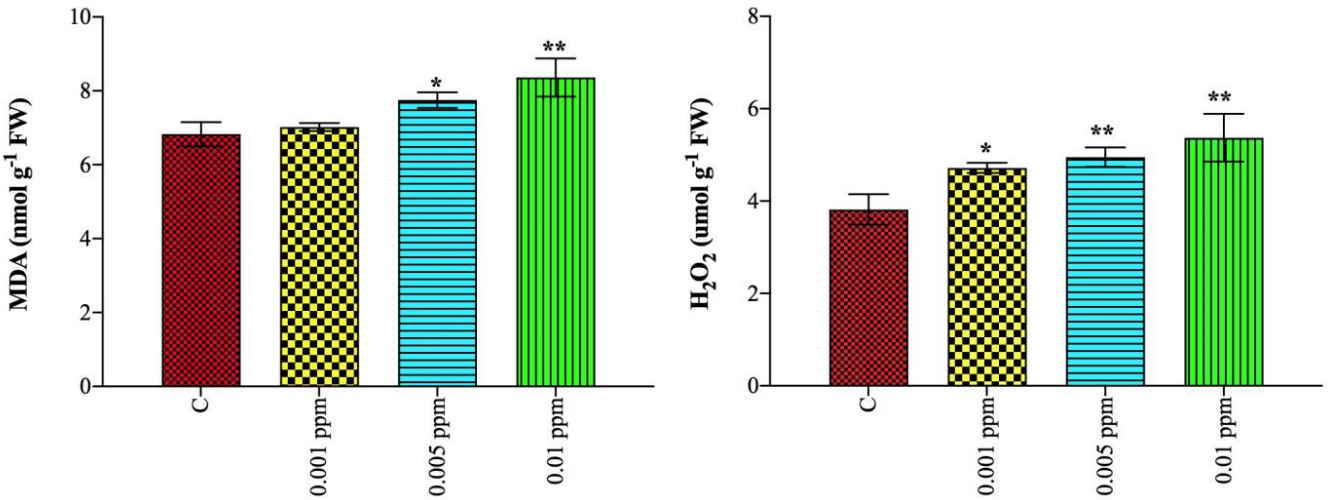

Fig. 2. Effect of deltamethrin on MDA and H_2O_2 levels in *L. minor*. Values are given as mean ± S.D. *p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001.

Compared to the control group, deltamethrin at a concentration of 0.001 ppm did not change the MDA content in *L. minor* (Fig. 2). On the other hand, deltamethrin applied to *L. minor* at concentrations of 0.005 and 0.01 ppm significantly increased the MDA content compared to the control. In addition, deltamethrin applied to *L. minor* significantly increased the H_2O_2 content depending on the dose increase. This increase was approximately 40% in the 0.01 ppm group compared to the control (Fig. 2). These results showed that deltamethrin induced oxidative stress especially at high concentrations. In plants, reactive oxygen species (ROS) such as H_2O_2 are mainly produced in low amounts in different organelles. Under environmental stressors, ROS production affects the antioxidant system in plants and exceeds the antioxidant scavenging capacity, leading to oxidative stress. In parallel with our findings, it was reported that deltamethrin applied to tomato plants increased the amount of H_2O_2 (Touzout et al. 2021). As it is known, excessive production of ROS in plants demege cell membranes, disrupts membrane permeability and consequently prevents plant growth by negatively affecting the physiological activities of the plant. In our study, it was determined that especially high concentrations of deltamethrin increased the amount of MDA in *L. minor*. This was attributed to the H_2O_2 production induced by deltamethrin causing lipid peroxidation. Consistent with our results, deltamethrin was reported to increase MDA in maize and tomato (Duran et. al. 2015; Touzout et al. 2021).

Exposure to different concentrations of deltamethrin significantly affected the activities of SOD, CAT, POD, APX, GR, GPX, GST, MDHAR and DHAR enzymes in *L. minor*. SOD, CAT and GST activities increased significantly with the dose increase of deltamethrin (Fig. 3, Fig. 4), while APX, GPX and MDHAR activities decreased significantly in a dose-dependent manner (Fig. 4 and Fig. 5). While POD activity did not change with 0.001 ppm deltamethrin application compared to the control group, it increased with increasing dose (Fig. 3). GR activity decreased significantly in the 0.001 ppm deltamethrin treated group, while GR activity did not show a significant change in the other treatment groups compared to the control (Fig. 4). DHAR activity increased in the 0.005 ppm group and decreased in the 0.01 ppm group (Fig. 5).

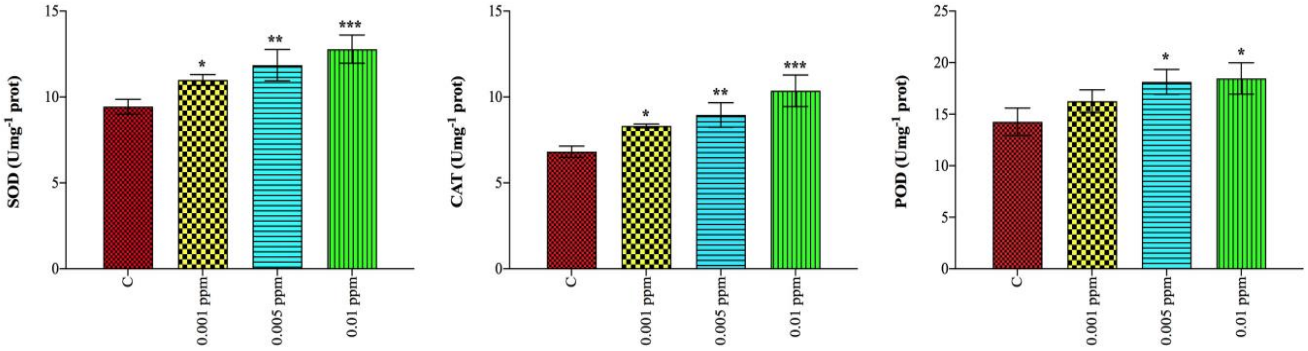

Fig. 3. Effect of deltamethrin on SOD, CAT, and POD enzyme activities in *L. minor*. Values are given as mean ± S.D. *p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001.

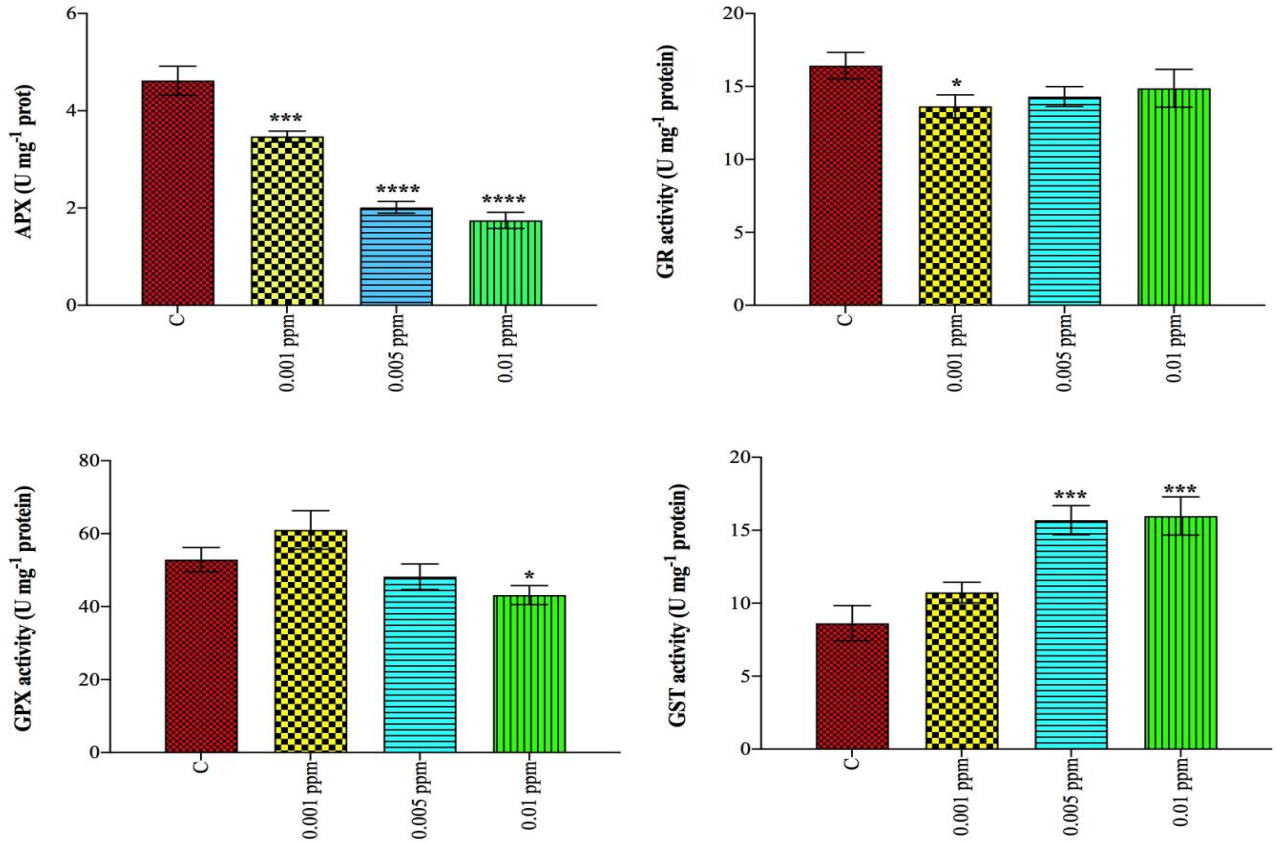

Fig. 4. Effect of deltamethrin on APX, GR, GPX, and GST enzyme activities in *L. minor*. Values are given as mean ± S.D. *p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001.

The increase of ROS in plants triggers a complex mechanism involving enzymatic (SOD, CAT, POD, APX, GR, GPX etc.) and non-enzymatic (ASA, GSH) systems to scavenge ROS. In plants, SOD functions as the first line of defense by catalyzing the conversion of $O_2^{\bullet-}$ to O_2 and H_2O_2. CAT, and POD are responsible for the scavenging of H_2O_2 and convert H_2O_2 to O_2 and H_2O. In our study, it was determined that the amount of SOD, CAT and POD increased parallel to deltamethrin administration (Fig. 3). Since these enzymes scavenge ROS, the increase in enzyme amounts with deltamethrin treatment is due to increased H_2O_2 accumulation. Similar to our results, deltamethrin was reported to increase SOD activity in soybean and CAT and POD activities in tomato (Bashir et al. 2007; Touzout et al. 2021). The amounts of ASA and GSH in deltamethrin-treated *L. minor* plants are given in Figure 5. Exposure to low doses of deltamethrin increased both ASA and GSH levels (Fig. 5). On the other hand, deltamethrin applied to *L. minor* at a concentration of 0.01 ppm decreased the amounts of ASA and GSH (Fig. 5). APX activity, one of the ASA-GSH cycle enzymes, decreased significantly with increasing deltamethrin dose. GR activity decreased in the 0.001 ppm treated group, but did not change in the 0.005 and 0.01 ppm treated groups compared to the control group. GPX, MDHAR and DHAR activities were increased by low doses of deltamethrin and significantly decreased in 0.01 ppm treated groups compared to the control. GST activity increased with increasing dose.

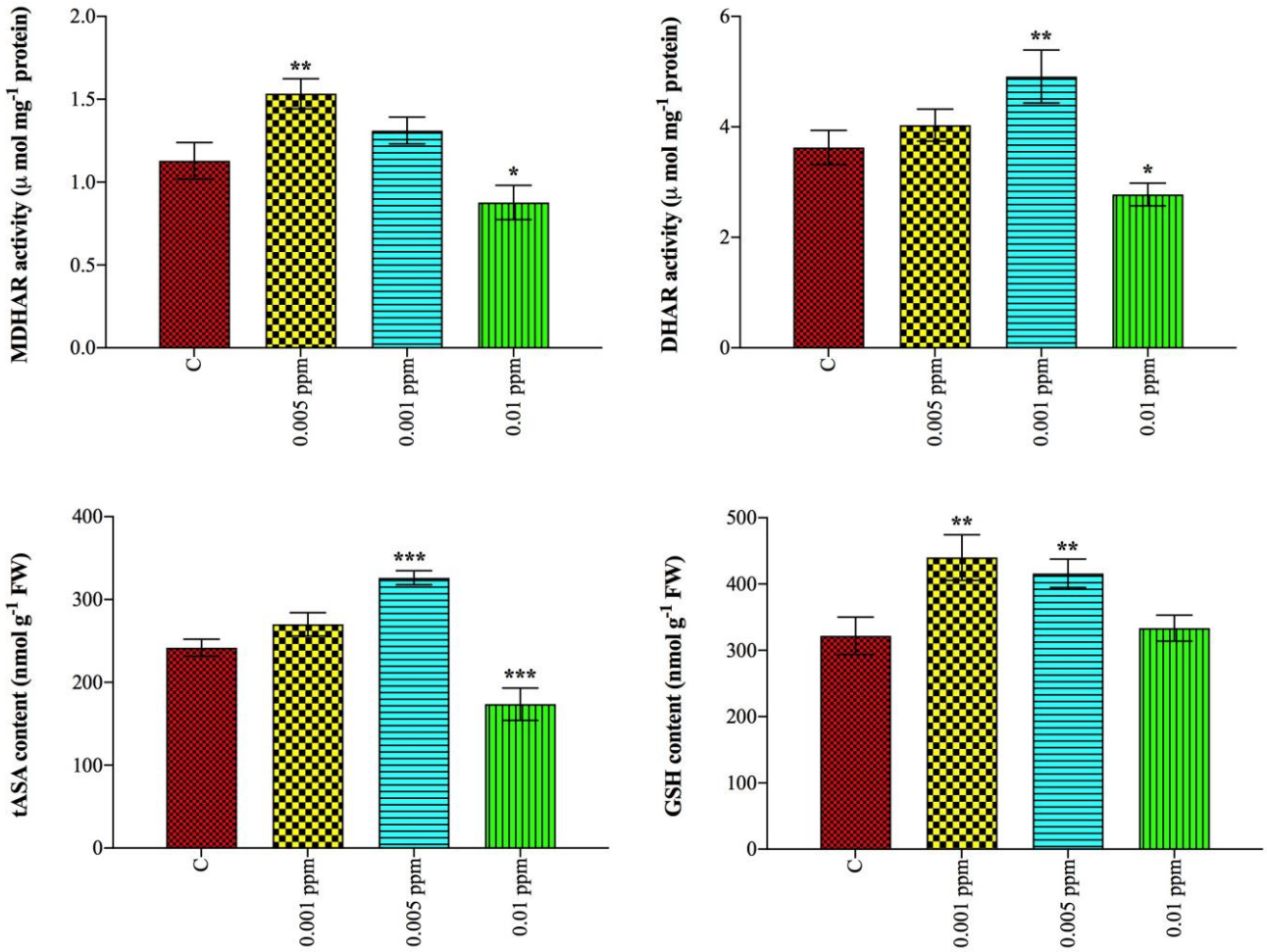

Fig. 5. Effect of deltamethrin on MDHAR and DHAR enzyme activities and ASA and GSH levels in *L. minor*. Values are given as mean ± S.D. *p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001.

ASA and GSH are the primary soluble antioxidants that protect plants from various environmental stressors. These antioxidants protect cells against ROS and maintain cellular redox balance. The amounts of these antioxidants increased in *L. minor* plants exposed to low dose (0.01 ppm) of deltamethrin. Similar to our results, low doses of deltamethrin increased the amounts of ASA and GSH in tomato and pea plants exposed to deltamethrin (Bashir et al. 2007; Touzout et al. 2021). The defense function of ASA against pesticide-induced oxidative stress in plants has been reported in many studies. It has also been reported that GSH eliminates reactive oxygen species in plants. In addition, GSH has been reported to protect cell membrane proteins and lipids against oxidation by inducing GST activity. In this context, in our study, it was also found that GST activity increased due to deltamethrin treatment. On the other hand, the ASA-GSH cycle is maintained by the activities of APX, GR, MDHAR and DHAR enzymes. Exposure to high doses of deltamethrin suppressed the activities of these enzymes, leading to a decrease in ASA and GSH levels and loss of redox status. In this case, it can be said that high doses (0.01 ppm) of deltamethrin applied to *L. minor* triggered oxidative stress and antioxidant enzymes and ASA-GSH cycle were ineffective in scavenging excess ROS.

Conclusions

As a result, the amount of photosynthetic pigments decreased and MDA and H_2O_2 levels increased in parallel with the increase in deltamethrin concentration. Deltamethrin exposure induced oxidative stress through ROS accumulation and led to disruptions in membrane structure by increasing lipid peroxidation. Increasing deltamethrin doses increased SOD, CAT and GST enzyme activities. This was interpreted as a defense mechanism to eliminate H_2O_2 accumulated in the plant. However, the activities of ASA-GSH cycle enzymes such as APX, GPX, GR, MDHAR and DHAR decreased at high doses, resulting in disruption of redox balance and increased oxidative stress. Low dose deltamethrin (0.001 ppm) treatment increased ASA and GSH amounts and supported the resistance of plants against oxidative stress. However, deltamethrin at 0.01 ppm concentration decreased the levels of these antioxidants and decreased ROS scavenging capacity. In conclusion, at low doses, deltamethrin activated some defense mechanisms to adapt to plant metabolism, but at high doses it increased oxidative stress and negatively affected the functionality of the antioxidant system. Our study showed that deltamethrin can cause loss of

photosynthetic pigment, lipid peroxidation and disruption of redox balance in plants. These findings are important for understanding the negative effects of pesticides on aquatic biota and promoting conscious use in the field of environmental toxicology.

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Authors' contributions: OA: Design, data analysis, manuscript writing, laboratory experiments

HU: Manuscript writing, EG: Laboratory experiments

Conflict of interest disclosure:

The authors declare no conflict of interest.

References

Abdelkhalek NKM, Ghazy EW, Abdel-Daim MM. (2015) Pharmacodynamic interaction of Spirulina platensis and deltamethrin in freshwater fish *Nile tilapia*, Oreochromis niloticus: impact on lipid peroxidation and oxidative stress. Environ Sci Pollut Res 22:3023–3031.

Agarwal S, Pandey V. (2004) Antioxidant enzyme responses to NaCl stress in *Cassia angustifolia*. Biol Plant 48, 555-560.

Alp FN, Arikan B, Ozfidan-Knakcı C, Gelentürk C, Yildiztugay E, Turan M, Cavusoglu H. (2023) Hormetic activation of nano-sized rare earth element terbium on growth, PSII photochemistry, antioxidant status and phytohormone regulation in *Lemna minor*. Plant Physiol Biochem 194:361-373.

Barlow SM, Sullivan FM, Lines J. (2001) Risk assessment of the use of deltamethrin on bednets for the prevention of malaria. Food Chem Toxicol 39:407-422.

Bashir F, Siddiqi TO, Iqbal M. (2007) The antioxidative response system in *Glycine max* (L.) Merr. exposed to deltamethrin, a synthetic pyrethroid insecticide. Environ Pollut 147:94–100.

Duran RE, Kilic S, Coskun Y. (2015) Response of maize (*Zea mays* L. saccharata Sturt) to different concentration treatments of deltamethrin. Pestic Biochem Phys 124:15-20.

Foyer CH, Halliwell B. (1976) The presence of glutathione and glutathione reductase in chloroplasts: a proposed role in ascorbic acid metabolism. Planta 133:21-25.

Gong Y, Toivonen PMA, Lau OL, Wiersma PA. (2001) Antioxidant system level in '*Braeburn*' apple in related to its browing disorder. Bot Bul Acad Sin 42:259-264.

Habig WH, Pabst MJ, Jakoby WB. (1974) Glutathione S-transferases: the first enzymatic step in mercapturic acid formation. J Biol Chem 249(22):7130-7139.

Hasanuzzaman M, Nahar K, Alam MM, Fujita M. (2012) Exogenous nitric oxide alleviates high temperature induced oxidative stress in wheat (*Triticum aestivum* L.) seedlings by modulating the antioxidant defense and glyoxalase system. Aust J Crop Sci 6(8):1314-1323.

Huang C, He W, Guo J, Chang X, Su P, Zhang L. (2005) Increased sensitivity to salt stress in an ascorbate-deficient Arabidopsis mutant. J Experiment Bot 56:3041-3049.

Kumar A, Sasmal D, Bhaskar A, Mukhopadhyay K, Thakur A, Sharma N. (2016) Deltamethrin-induced oxidative stress and mitochondrial caspase-dependent sig- naling pathways in murine splenocytes. Environ Toxicol 31:808–819.

Li R, Luo C, Qiu J, Li Y, Zhang H, Tan H. (2022) Metabolomic and transcriptomic investigation of the mechanism involved in enantioselective toxicity of imazamox in *Lemna minor*. J Hazard Mater 425:127818.

Lu Q, Sun Y, Ares I, Anadón A, Martínez M, Martínez-Larrañaga M-R, Yuan Z, Wang X, Martínez M-A. (2019) Deltamethrin toxicity: A review of oxidative stress and metabolism. Environ Res 170:260-281.

Miyake C, Asada K. (1992) Thylakoid-bound ascorbate peroxidase in spinach chloroplasts and photoreduction of its primary oxidation product monodehydroascorbate radicals in thylakoids. Plant Cell Physiol 33:541-553.

Nakano Y, Asada K. (1981) Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. Plant Cell Physiol 22:867-880.

OECD. (2006) OECD guidelines for the testing of chemicals, revised proposal for a new guideline 221, Lemna sp. Growth inhibition test. OECD, Paris.

Touzout N, Mehallah H, Moralent R, Nemmiche S, Benkhelifa M. (2021) Co-contamination of deltamethrin and cadmium induce oxidative stress in tomato plants (*Solanum lycopersicum* L.). Acta Physiol Plant 43:91.

WHO (World Health Organization). (2010) Specifications and evaluations for public health pesticides- deltamethrin. World Health Organization, Geneva.

Yee Y, Tam NFY, Wong YS, Lu CY. (2002) Growth and physiological responses of two mangrove species (*Bruguira gymnorrhiza* and *Kandelia candel*) to waterlogging. Environ Experiment Bot 1:13.

Yu C-W, Murphy TM, Lin C-H. (2003) Hydrogen peroxide-induced chilling tolerance in mung beans mediated through ABA-independent glutathione accumulation. Funct Plant Biol 30: 955-963.